AD-773 902

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M. G. Kichenko, et al

Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

15 January 1974

FTD-HT-23-519-74

FOREIGN TECHNOLOGY DIVISION

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M. G. Kichenko, Yu. G. Talayeva

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Foreign Technology Division
Air Force Systems Command
U. S. Air Force

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
A METHOD FOR RAPID IDENTIFICATION OF TYPHOID AND DYSENTERY BACTERIA IN WATER

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Translation

5. AUTHOR(S) (First name, middle initial, last name)
M. G. Kichenko, Yu. G. Talayeva

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
1965	89	

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. JDX4	FTD-HT-23-519-74
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10 DISTRIBUTION STATEMENT
Approved for public release;
distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Foreign Technology Division Wright-Patterson AFB, Ohio

13. ABSTRACT
06

DD FORM 1 NOV 68 1473

Unclassified
Security Classification

EDITED TRANSLATION

FTD-HT-23-519-74 15 January 1974

AT8015323

A METHOD FOR RAPID IDENTIFICATION OF TYPHOID AND DYSENTERY BACTERIA IN WATER

By: M. G. Kichenko, Yu. G. Talayeva

English pages: 6

Source: Akademiya Meditsinskikh Nauk SSSR, Voprosy Sanitarnoy Bakteriologii i Virusologii, 1965, pp. 166-170

Country of Origin: USSR
Translated by: MSgt Victor Mesenzeff
Requester: FTD/PDTR/D. G. Matheson
Approved for public release; distribution unlimited.

A METHOD FOR RAPID IDENTIFICATION OF TYPHOID AND DYSENTERY BACTERIA IN WATER

M. G. Kichenko and Yu. G. Talayeva
(Institute of General Communal Hygiene im. A. N. Sysin AMS USSR)

It is known that today the antiepidemic measures, to a considerable extent, depend on the speed with which the causative agent of an infectious disease is detected. The present level of development in microbiology and other related areas of science permits one to consider the stating of the problem concerning further extensive development of rapid identification methods for pathogenic bacteria in an external medium, improvement of the existing methods and implementing them into practice as timely.

Up until now the bacteriological methods described in literature for direct identification of pathogenic bacteria of the enterobacteriaceae family in water can be divided into three basic stages: concentration of bacteria obtained from water, culturing on nutrient media and isolation of a pure culture and, finally, identification of the obtained pure cultures, using biochemical, serological and other means. The entire procedure lasts about 72 h, which cannot satisfy the demands of the practice. In view of this fact many authors propose various methods for speeding up a particular stage in the analysis of water for the presence of

pathogenic bacteria or completely replace the direct determination of bacteria by indirect metnods which indicate the level of seeding of the water.

We decided to solve the problem regarding speeding up of the analysis by reducing the time in a number of stages during the study. Thus,it has been established that among the well-known methods for concentrating bacteria from water (filtration through diatomaceous earth, centrifugation, settling by means of the various types of coagulants, filtration through membrane filters, electrical precipitation, bacteria-trapping method of Berezov) the most improved is the one where bacteria are concentrated via filtration through membrane filters and filters made from new synthetic materials, for example, filters of the type FPP-5. M. G. Kichenko has checked the properties of the new filtering materials with regard to the growth on them of pathogenic bacteria of the enteric group and compared them with the membrane filters. Good positive characteristics of these new materials have been established, especially for the FPP-5, for the bacteriological practice since rapid and relatively complete trapping of microorganisms from water is achieved not only as a result of immediate retantion of bacteria in the filter pores, but also due to the electric precipitation phenomenon. The advantage of this bacteria concentration method consists also of the fact that the bacteria isolated from water can be grown on liquid or solid nutrient media without preliminary processing. At the present time membrane filters are being widely used in sanitary microbiology.

Obviously, the most important stage in bacteriological analysis is the accumulation of a biological mass of pathogenic bacteria by growing them on nutrient media, since pathogenic bacteria in external medium constitute a negligible small percent as compared with the saprophytic flora existing in external medium, especially with the coliform bacteria. In view of this

fact high specifications are imposed on nutrient medium: it should be able to quickly inhibit the growth of an accompanying microflora and facilitate a maximum growth of the pathogenic bacteria being sought. The existing elective nutrient media do not meet these requirements. For example, the widely known Wilson-Blair medium used for growing pathogenic bacteria of the enteric group strongly inhibits the growth of saprophytic microflora, but, at the same time, it also inhibits the growth of the pathogenic bacteria to a considerable degree: they have to be incubated not for 24 h, but for 48 h.

It is known that the growth of pathogenic bacteria is improved by planting on the nutrient medium surface bacteria which were concentrated by the membrane filter. In all probability the extensive seeding is conducive in diminishing the antigonistic influence of the accompanying microflora on the growth of the pathogenic bacteria, improves the accessibility to the nutrient substances and intensifies diffusive processes into the nutrient medium for the products needed for vital activity of the bacteria.

The density of nutrient medium and incubation temperature also have a considerable effect on the speed with which the biological mass is accumulated. In our laboratory we grew bacteria on a semisolid media, readily visible with a naked eye just after 8-10 h of incubation at 39-40°. The use of aeration of liquid nutrient media increases the processes of biomass accumulation considerably. With this in mind M. G. Kichenko used an electromagnetic mixer in her work for mixing the culture being grown in liquid nutrient medium.

The most effective growth-promoting factor for the pathogenic bacteria on semiliquid nutrient media proved to be the biostumulators obtained from oat shoots. The addition of the shoot extract to nutrient medium in the ratio of 2-3 ml of extract to 10-12 ml of medium has enabled us to obtain the quickest growth of the pathogenic bacteria in short time (8-10 h).

However, the methods mentioned above do not contribute principally new approaches toward the solution of this very important and complex problem - obtaining a rapid growth of the pathogenic bacteria isolated from water in the presence of the accompanying saprophytic microflora. In our opinion, in solving the problem with regard to the highly elective nutrient media, the most promising is the biochemical aspect which is based on the study of bacteria's metabolism. We think that finding the optimum media for the pathogenic bacteria of various groups is one of the most promising methods for obtaining a rapid growth of the pathogenic bacteria in mixed cultures.

The third stage in the study of the bacteria isolated from water is the verification of biochemical activity by inoculating the differential nutrient medium on the so-called variegated series. The results of the inoculation are examined after 20-24 h. This stage of bacteriological analysis, as is known, can also be shortened by inoculating the material in question on small amounts of differentiating media which are preheated to 39°, containing an increased amount of the indicator for obtaining more distinct changes in color when there is a slight change in the pH of the medium. Already, as early as 3-5 h after inoculation, we are able to obtain relatively clear results regarding the biochemical activity of the studied cultures.

A further identification of cultures is usually accomplished by means of serological characteristics. The most complete and exact serological characteristic, which indicates as to the type of bacteria in question, is obtained by an extended agglutination which takes about 18-24 h to complete. Various methods which reduce the time of this reaction are also known. The most interesting of them is the method where fluorescent sera are used for microagglutination in pure and mixed cultures.

For the past few years our laboratory has been working on the possible use of fluorescent sera for an early rapid diagnosis of pathogenic bacteria of the coliform group. The results of these studies permit us to conclude that good results are obtained in working with pure cultures; in mixed cultures, with which we usually deal when isolating pathogens from water, there are instances of nonspecific fluorescence of bacterial cells, for example of paracolon, which is verified also by other authors. In our opinion the use of fluorescent sera for rapid identification of bacteria is very promising only with regard to the groups of organisms with clearly defined antigen structure, for example, for Vibrio cholerae, plague bacilli, anthrax bacilli and other causative agents of particularly dangerous infections. However, the pathogens of the Enterobacteriaceae family are very similar in their antigenic nature ot the various types of saprophytic bacteria; thus, fluorescent sera can be of limited use as a signaling rapid method for determining pathogenic bacteria with a mandatory more extensive further study of the biochemical and other properties of the culture.

A combination of methods indicated above for shortening bacteriological analysis of water for the presence of pathogens makes it possible for one to obtain results not in 72 h, as usually, but in 18 h.

At the present time, in addition to the direct methods for isolating pathogens from water, indirect methods for determining the presence of pathogens in an external medium are being used more and more. These methods are based on the use of the different properties of bacteria or peculiarities of their chemical composition. Thus, the precipitation reaction with hapten in a mixed culture, proposed by E. B. Steiman and Ye. D. Ravich-Berger, is based on the multisugar complex of pathogens to give a precipitative reaction with specific sera. As an indirect index of infectiousness of the studied material, V. D. Timakov and D. M. Goldfarb proposed the use of the ability of a specific (indicating)

bacteriophage to multiply only in cells of the corresponding group of pathogens.

However, these direct methods do not preclude the need for a further development of rapid bacteriological methods for direct isolation of pathogenic bacteria from water, and also their further study in order to solve the problem concerning the epidemilogical danger of the strains isolated from water.